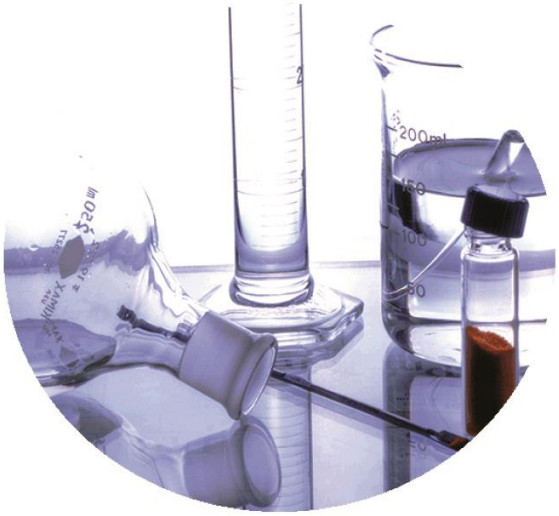

Assay kits

# ONPG Assay Kit

ONPG Galactosidase Assay Kit

# Protocol

OZBIOSCIENCES
The art of delivery systems

# IMPORTANT NOTES – Before you begin

Ready-to-use assay system for quantitatively measuring □-galactosidase expression levels in transfected cells using ONPG as a sensitive substrate.

- ✓ LacZ is one of the most frequently used reporter gene in transfection experiments because the gene product specific properties. Indeed, the LacZ encoded protein, β-galactosidase, is very stable, resistant to proteolytic degradation and easily tested. The levels of active β-galactosidase expression can be quickly measured by its catalytic hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG) substrate to a bright yellow product.
- ✓ All the necessary reagents provided in this assay kit offer a rapid, simple and sensitive method to quantify the enzyme expression level in transfected cells.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

# ONPG -Galactosidase Assay Kit | Specifications

| | |
|---|---|
| Package content | GO10001<br>5X Lysis Buffer (55 ml)<br>Standard Dilution Buffer (55 ml)<br>Substrate Buffer (55 ml)<br>Stop Buffer (55 ml)<br>β-gal enzyme standard, 40 units (100µl)<br>The kit is provided with sufficient reagents to perform 500 micro assays in 96-well plate. |
| Shipping conditions | The kit is shipped with gel pack (4◦C) |
| Storage conditions | Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions<br>5X Lysis Buffer (+4°C)<br>Standard Dilution Buffer (+4°C)<br>Substrate Buffer (+4°C)<br>Stop Buffer (+4°C)<br>β-gal enzyme standard, 40 units (-20°C) |
| Shelf life | 1 year from the date of purchase when properly stored and handled |
| Important notice | For research use only. Not for use in diagnostic procedures. |

## 1. Usage

1. Transfect cells with a plasmid expressing Lac Z gene
2. Lyse the cells using the lysis buffer
3. Transfer the lysate to a fresh tube or a micro titer plate. Dilute the lysate if needed
4. Prepare a β-galactosidase standard curve with standard dilution buffer
5. Add the substrate and monitor the color development at 405 nm - 420 nm
6. Calculate the expression levels based on a standard curv

## 2. General Conditions

- Before use, dilute the 5X Lysis buffer to 1X with distilled water. The surplus of unused 1X Lysis Buffer may be stored at +4°C for future use.
- The ONPG substrate buffer is ready-to-use. **CAUTION**: Wear Gloves for manipulating the ONPG since it will stain exposed skin.

## 3. General Protocol

- **Harvesting adherent cells:**

1. Aspirate the growth medium 24-72 hours after transfection from the culture dish including the control cells (non-transfected). Cells can be optionally washed once with 1X PBS.
2. Add 1X Lysis Buffer to the culture dish. Solution volumes recommended for various plates are:

| Type of culture dish | Volume of 1X Lysis Buffer (µL / well) |
|---|---|
| 96-well plate | 50 |
| 24-well plate | 250 |
| 12-well plate | 500 |
| 6-well plate | 1000 |
| 60 mm dish | 2500 |
| 100 mm dish | 5000 |

3. Incubate the dish 10-15 min. at room temperature by swirling it slowly several times to ensure complete lysis. The dishes can be observed under a microscope to confirm the complete lysis.

**NOTE**: A fast freeze/thaw cycle (freeze 1-2 hours at –20°C or -70°C and thaw at room temperature) of the dish can also be done to achieve a good lysis. Proceed to the colorimetric assay or freeze the plate at –70°C until ready.

4. **OPTIONAL**: The dish can be centrifuged for 2-3 minutes to pellet the insoluble material before proceeding to the colorimetric assay. Then, the supernatant is ready to be tested.

- ***Harvesting suspension cells:***

1. 24-72 hours post-transfection, centrifuge the cells at 250 x g for 5 minutes. Then, aspirate the supernatant. Cells pellet can be optionally washed once with 1X PBS.

2. Resuspend the cell pellet in 1X Lysis Buffer. The amount of Lysis Buffer depends on the size of the culture dishes used for transfection (i.e., cell pellet size) and we recommend using between 50 to 2000µL.

3. Incubate the cell lysate 10-15 min. at room temperature by gently swirling the dishes several times to ensure complete lysis. Proceed to the colorimetric assay or freeze the plate at –70°C until ready.
   **NOTE**: A quick freeze/thaw cycle (freeze 1-2 hours at –20°C or –70°C and thaw at room temperature) can also be done to obtain a good lysis.
4. **OPTIONAL**: The dish can be centrifuged 2 min. to pellet the insoluble material before proceeding to the colorimetric assay. Then, the supernatant is ready to be assayed.

### 4. 96 well micro titer plate assay*

1. If needed thaw the dish, tube or plate of lysed cells at room temperature. If the transfection is performed with a 96-well plate, perform the assay directly on the plate.
2. Add 50 µl of Standard Dilution Buffer to the wells of a 96-well plate (flat bottom) except the control wells, which are save for the standard curve.
3. Prepare a serial dilution of β-galactosidase standard in Standard Dilution Buffer. Then, transfer 50 µL of each point of the standard curve to the control wells (containing 50µL of cell lysate). A dilution protocol for an optimized standard curve is suggested in the following table :

| β-Gal Standard (milliunits) | Standard Dilution Buffer Volume | β-Gal Standard Volume |
|---|---|---|
| 200 | 990 µL | 10 µL of β-gal standard stock |
| 100 | 200 µL | 200 µL of 200 mu β-gal standard |
| 50 | 200 µL | 200 µL of 100 mu β-gal standard |
| 25 | 200 µL | 200 µL of 50 mu β-gal standard |
| 12.5 | 200 µL | 200 µL of 25 mu β-gal standard |
| 6.25 | 200 µL | 200 µL of 12.5 mu β-gal standard |
| 3.125 | 200 µL | 200 µL of 6.25 mu β-gal standard |
| 1.562 | 200 µL | 200 µL of 3.125 mu β-gal standard |

**NOTE 1**: Adjust the standard curve to suit the specific experimental conditions, such as cell type, cell number, transfection reagent, size of the culture dish or plasmid vector.

**NOTE 2**: The dilutions for the standard curve must be prepared freshly each time the assay is performed.

1. Add 50 µL of each sample/well.
   **NOTE**: It may be necessary to dilute the lysate in 1X Lysis Buffer when transfection efficiency is very high. In contrast, when transfection efficiency is low, reduce the volume of lysis buffer used to harvest the cells (see above) or use up to 150 µL of cell lysate for the colorimetric assay. If the transfection is performed with a 96-well plate, perform the assay directly on the plate.

   To control endogenous β-galactosidase activity, prepare controls (blank) by adding 50 µL of lysis buffer to a well and 50 µL of cell lysate from non-transfected cells to another well.
2. Add 100 µL of ONPG Substrate buffer to each well. Incubate the plate at room temperature until the yellow color develops (from approximately 10 min. to 4 h depending on the cell type).
3. Read the absorbance at 405-420 nm with a micro titer spectrophotometer. Stop solution is not required for this format, since all wells are read simultaneously. Avoid the presence of bubbles in the wells while reading. Bubbles will interfere with the absorbance reading and can be removed with a fine gauge needle, tips or very weak gas flow.
4. Quantify β-galactosidase expression based on a linear standard curve.

*Felgner, J.H. *et al.* Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J. Biol. Chem.* **269**, 2550-2561 (1994)

## 5. Macro assay

1. Thaw the cell lysate (if needed) and transfer 100 µL to a fresh tube, or 50 µL to a 96-well plate. If a 96-well plate is used, follow the protocol described above.
   **NOTE**: It may be necessary to dilute the cell lysate in 1X Lysis Buffer when transfection efficiency is very high. In contrast, when transfection efficiency is very low, reduce the volume of lysis buffer used to harvest the cells (see description above) or use up to 150 µL of cell extract for the colorimetric assay

   Prepare a blank by adding 100 µL of lysis buffer to a tube. Add also 100 µL of cell lysate from non-transfected cells (mock-transfected cells) to a tube to control endogenous β-galactosidase activity.
2. Add 50 µL of Standard Dilution Buffer to each tube.
3. Prepare a serial dilution of β-galactosidase (E. Coli) standards with Standard Dilution Buffer separately. Transfer 50 µL of each standard to a fresh tube containing 100 µL cell lysate from non-transfected cells. The highest recommended amount of beta-galactosidase is 400,000 pg. (200 milliunits). Adjust the standard curve to suit the specific experimental conditions, such as cell type, transfection reagent, or plasmid vector. 2X serial dilution of standard curve consisting of 8 points is recommended. A dilution protocol example is shown in the section of 96-well plate assay.
4. Add 300 µL of the ONPG Substrate buffer to each tube. Incubate the tubes at room temperature until the yellow color develops (from approximately 10 minutes to 4 hours depending on the cell type). Add 500 µL of Stop Solution to stop the reaction. Final volume is 950 µL.
5. Read the absorbance at 405-420 nm with a spectrophotometer.
6. Quantify β-galactosidase expression based on a linear standard curve.

## Additional products

- **CPRG assay kit** to quantify low expression level of beta-Galactosidase
- **X-Gal Staining kit** to visualize □-gal expression through hydrolysis of the X-Gal substrate yielding blue precipitates

**Purchaser Notification**

**Limited License**
The purchase of the ONPG -Galactosidase Assay Kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the ONPG -Galactosidase Assay Kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all ONPG -Galactosidase Assay Kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a ONPG -Galactosidase Assay Kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the ONPG -Galactosidase Assay Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

**Product Use Limitations**
ONPG -Galactosidase Assay Kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.


**EUROPE & ASIA**
**OZ Biosciences SAS**

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

**USA & CANADA**
**OZ Biosciences INC**

4901 Morena Blvd
Suite 901
San Diego CA 92117
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com



Rev. 04/2021 FB